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Chapter 7

Metabolic Disorders of Copper Metabolism

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1. Introduction

Copper, atomic number 29, is an extremely versatile element both outside and inside living cells. The malleability, ductility, and esthetic properties of copper have promoted its use in pottery, ornaments, and currency for centuries. During the more recent decades, the electrical conductivity of copper has had an important role in the advancement of electrical technology.

Whereas elemental copper adds to the comfort and enjoyment of life, ionic copper is absolutely essential for the maintenance of life. Probably because of copper's relative abundance in the biosphere and the element's ease of undergoing oxidation reduction, organisms have developed enzyme systems that utilize copper in their catalytic action. Copper-containing enzymes have been identified in nearly every type of organism, from the simplest to the most complex, and the chemical reactions catalyzed by these enzymes cover a broad spectrum of metabolic processes (Frieden *et al.*, 1965). Because copper is an integral component of a variety of enzymes, a deficiency of this element or an abnormality in the production of a copper enzyme may result in widespread deleterious effects, and often death. In addition, because copper is a very reactive element, an excess of this ion in living cells results in marked changes in metabolism.

1.1. Copper Homeostasis

A discussion of the metabolic disorders associated with copper deficiency and copper toxicity is probably best prefaced by a short, and hopefully up-to-

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date, review of our knowledge of the homeostatic mechanisms involved in the absorption, transport, storage, and excretion of copper in organisms subject to metabolic disorders of copper metabolism. Figure 1 is a schematic diagram of the molecular pathways traversed by copper ions in mammalian systems. In the intestinal lumen, copper binds to ligands, probably amino acids, before it enters the intestinal epithelial cell. The passage of copper through the intestinal cell to the blood is apparently regulated by a low-molecular-weight copper-binding protein, the synthesis of which is induced by copper (Evans and LeBlanc, 1976). After passing through the intestinal epithelial cells, copper is transported through the portal blood as a histidine–copper–albumin complex (Lau and Sarkar, 1971) to the liver.

The liver is the key organ in the metabolism of copper. In the hepatic cells, copper is stored, incorporated into ceruloplasmin, and excreted from the body through the bile (Hazelrig *et al.*, 1966). The hepatic copper-storage compartment apparently consists of a low-molecular-weight copper-binding protein (Evans *et al.*, 1975; Riordan and Gower, 1975; Winge *et al.*, 1975), and the synthesis of

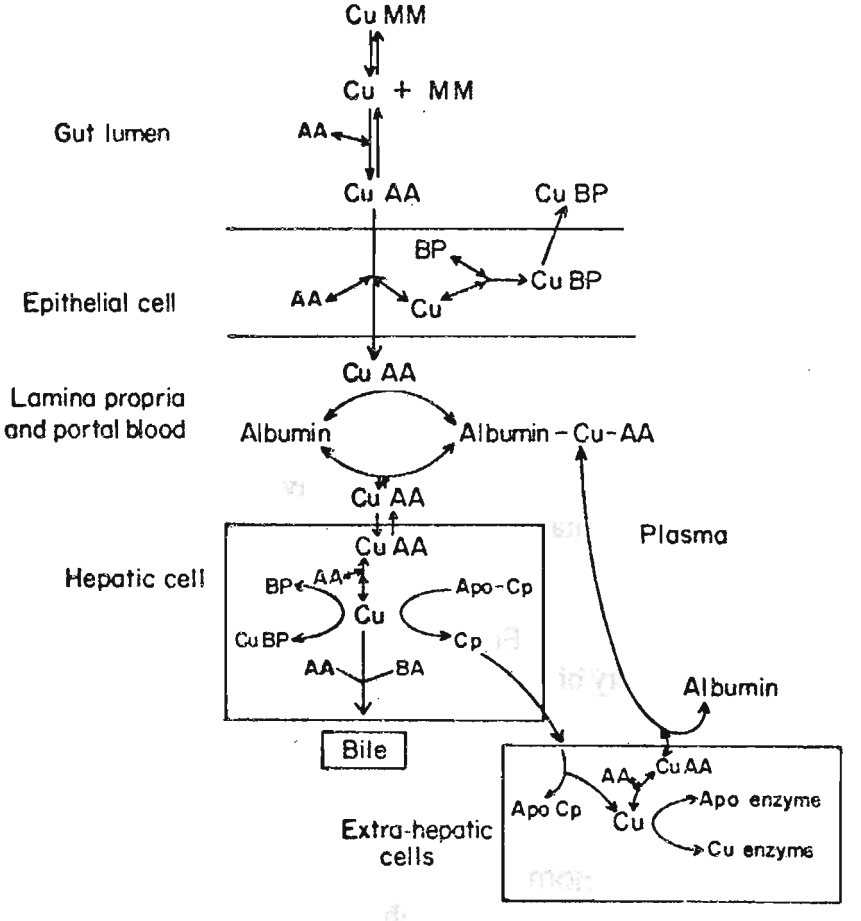

Fig. 1. Schematic illustration of copper homeostasis in mammalian systems. Abbreviations: MM, macromolecule; AA, amino acid; BP, copper-binding protein; BA, bile acid; Cp, ceruloplasmin.

this protein is regulated by the copper level of the cell (Premakumar *et al.*, 1975). Copper apparently is excreted into the bile in the form of amino acid (Evans and Cornatzer, 1971) and biliary acid complexes (Lewis, 1973) that combine with macromolecules, rendering the copper unavailable for reabsorption (Mistilis and Farrer, 1968).

The incorporation of copper into ceruloplasmin is a vital function of the liver because copper is transported to the extrahepatic tissues in the form of ceruloplasmin. Several investigations have demonstrated that the appearance of labeled copper in extrahepatic tissues coincides with the disappearance of the label from ceruloplasmin in the plasma (Owen, 1965, 1971; Marceau and Aspin, 1972). Moreover, radioactive copper from ceruloplasmin is incorporated into hepatic and brain cytochrome c oxidase (Marceau and Aspin, 1973), and ceruloplasmin has been shown to be extremely effective in restoring cytochrome oxidase activity in tissues from copper-deficient animals (Hsieh and Frieden, 1975). The copper from ceruloplasmin, which is probably taken up by pinocytosis, is apparently more readily available to certain compartments in extrahepatic tissues than copper from albumin in spite of the fact that albumin copper is easily dissociated.

The pathways outlined above indicate that a variety of ligands are involved in the transport of copper from the intestine to the organs of the body. Once within the cells of the various organs, copper is incorporated into the enzymes (Table I) that require the element for catalytic activity. Thus, metabolic disorders of copper metabolism can be classified into three categories: (1) disorders that result from a lack of dietary copper or unavailability of dietary copper caused by a high level of copper antagonists in the food; (2) disorders that result from inborn errors of metabolism that affect the ligands involved in the absorption, transport, storage, or excretion of copper; and (3) inborn errors of metabolism that affect a specific copper-dependent enzyme. The discussion in the next section is organized according to these three categories.

Table I. Copper Enzymes in Mammalian Tissues

Enzyme	Reference
Benzylamine oxidase	Buffoni and Blaschko (1964)
Ceruloplasmin (ferroxidase)	Holmberg and Laurell (1948) Osaki *et al.* (1966)
Cytochrome c oxidase	Peisach *et al.* (1966)
Diamine oxidase, histaminase	Mondovi *et al.* (1967)
Dopamine β-hydroxylase	Friedman and Kaufman (1965)
Lysyl oxidase	Siegel *et al.* (1970)
Spermine oxidase	Yamada and Yasunobu (1962)
Superoxide dismutase	McCord and Fridovich (1969)
Tryptophan-2,3-dioxygenase	Brady *et al.* (1972)
Tyrosinase	Pomerantz (1963)
Uricase	Mahler *et al.* (1956)

2. Disorders Resulting from a Lack of or Unavailability of Dietary Copper

Copper deficiency occurs in animals restricted to pastures on copper-deficient soil (Underwood, 1971) and in humans restricted to low-copper milk diets (Cordano *et al.*, 1964) or total parenteral hyperalimentation (Karpel and Peden, 1972; Dunlap *et al.*, 1974). In addition, the manifestations of dietary copper deficiency occur in animals that consume diets that contain copper antagonists, such as sulfide ion, molybdenum, zinc, silver, mercury, and cadmium (Evans, 1973). The extent and types of disorders associated with copper deficiency depend on species, age, sex, and severity of deficiency, but the disorders generally include anemia; achromotrichia; lesions of the cardiovascular system, lung, skeleton, and central nervous system; changes in the growth and appearance of hair, fur, or wool; impaired growth; and reproductive failure.

2.1. Anemia

Copper has the distinction of being the second trace element (iron was the first) discovered to be essential in mammals; this discovery emerged from experiments in rats suffering from milk anemia. In 1928, Hart *et al.* (1928) observed that an anemia developed in rats fed a diet of milk that could not be ameliorated by iron supplements alone. When both copper and iron were added to the milk diet, the hemoglobin levels of the experimental animals were restored to normal. These observations provided the first evidence that copper is an essential nutrient, and the experiments that were confirmed and extended in other species forged the first link between iron and copper metabolism.

Following the announcement of Hart *et al.* (1928), several hypotheses were proposed to explain the role of copper in iron metabolism, but very little convincing and reproducible evidence appeared until the late 1960s. Curzon and O'Reilly (1960) showed that the cupric ions of ceruloplasmin could catalyze the oxidation of ferrous ions. Later, Osaki *et al.* (1966) first proposed that ceruloplasmin promotes hematopoiesis by catalyzing the formation of ferritransferrin.

During the last decade, ample evidence, both *in vivo* and *in vitro*, has been presented to confirm the hypothesis of Osaki *et al.* (1966). Ragan *et al.* (1969) examined the effects of ceruloplasmin on iron mobilization in copper-deficient pigs supplemented with iron by intramuscular injection. The pigs were fed an iron- and copper-deficient milk diet until a severe copper deficiency developed, after which they were injected intravenously with pig ceruloplasmin, $CuSO_4$, or copper-deficient pig plasma. Ceruloplasmin injections resulted in a significant and rapid elevation of plasma iron, but $CuSO_4$ and copper-deficient pig plasma produced little or no change in plasma iron levels. In other studies with copper-deficient pigs, Roeser *et al.* (1970) demonstrated that a marked decrease in serum ceruloplasmin precedes a decrease in serum iron with an accumulation of iron in

the liver. These investigators also observed that the decreased serum iron levels could be restored to normal by injecting ceruloplasmin.

Evidence for the effective mobilization of iron by ceruloplasmin was observed *in vitro* by Osaki *et al.* (1971). Livers were removed from dogs and copper-deficient pigs after which the livers were perfused with solutions that contained ceruloplasmin, apotransferrin, HCO_3, $CuSO_4$, glucose, fructose, citrate, or copper albumin. Ceruloplasmin was the only compound tested that had any appreciable effect on the mobilization of iron from the liver to the perfusate and the concentration of ceruloplasmin required to produce an effect was less than 1% of the normal ceruloplasmin concentration in plasma.

The experiments discussed above, as well as others of a similar nature (Frieden and Hsieh, 1976), indicate that ceruloplasmin is the long sought after link between copper and iron metabolism. For normal hemoglobin production to proceed, iron must be mobilized from reticuloendothelial cells to bone marrow cells. Transferrin, which binds ferric iron, is the only known protein that supplies iron to the marrow cells. Iron in cells is bound to ferritin, but the iron is released from ferritin in the ferrous state, which necessitates oxidation of the iron prior to its incorporation into apotransferrin. Because the uncatalyzed oxidation of ferrous iron is not sufficient to maintain normal hemoglobin production (Osaki *et al.*, 1966), ceruloplasmin, with copper incorporated in its structure, is essential to maintain hematopoiesis. Williams *et al.* (1974) have suggested that ceruloplasmin functions in iron mobilization as follows: (1) the ferrous iron released from ferritin binds to specific sites on reticuloendothelial cell membranes; (2) ceruloplasmin interacts with the iron-binding sites to form a ceruloplasmin–ferrous ion intermediate; and (3) iron is oxidized and transferred to apotransferrin by a specific ligand-exchange reaction (Fig. 2).

In summary, normal hemoglobin production depends on the ferroxidase activity of ceruloplasmin. Since the ferroxidase activity of ceruloplasmin is dependent on the presence of copper in the molecule, a deficiency of copper results in a marked decrease in ceruloplasmin ferroxidase activity, which leads to anemia.

2.2. Cardiovascular Defects

For several years after the announcement of Hart *et al.* (1928), the only known function for copper was its role in hematopoiesis. However, during the early 1960s several investigators began reporting experimental observations that indicated that copper had physiological functions more far-reaching that preventing anemia.

In 1961, Hill and Matrone (1961) observed a high mortality rate in copper-deficient chicks despite the fact that the anemia was not severe. Later, O'Dell *et al.* (1961) reported that the mortality observed in copper-deficient chicks is caused by rupture of the major blood vessels. O'Dell *et al.* (1961) presented

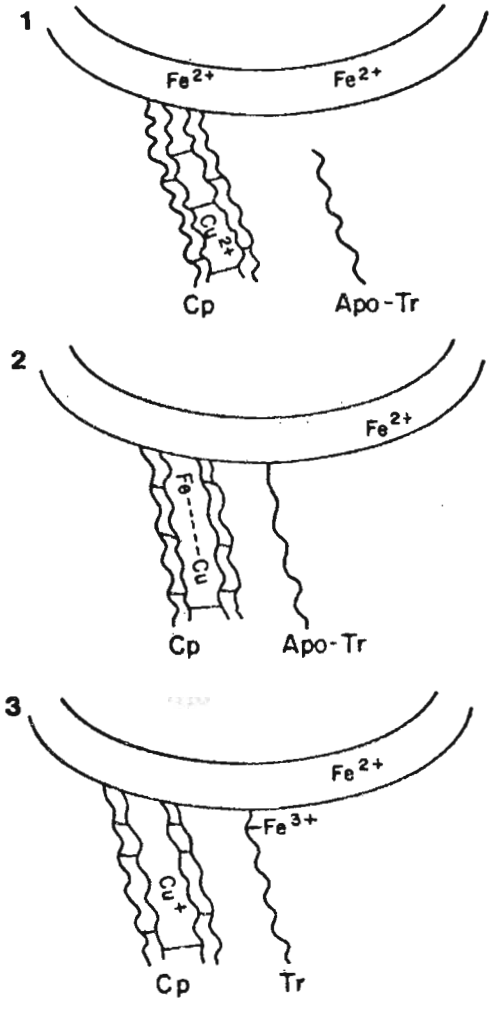

Fig. 2. Schematic illustration of ceruloplasmin (ferroxidase) function in iron mobilization. Abbreviations: Cp, ceruloplasmin; Tr, transferrin.

histological evidence that indicated that the elastic tissue in the aorta is abnormal in copper-deficient chicks.

Abnormal elastic membranes and death from aortic rupture are not limited to copper-deficient chicks. Coulson and Carnes (1963) and Shields *et al.* (1962) observed high mortality resulting from aortic rupture in pigs fed copper-deficient diets. These investigators also observed histological defects in the aortic elastic tissue from copper-deficient pigs.

The derangements observed in elastic tissue of blood vessels from copper-deficient animals prompted several investigators to examine the effect of copper deficiency on the synthesis of aortic elastin, the major component of elastic tissue. Weissman *et al.* (1963) and Kimball *et al.* (1964) isolated elastin from pig aorta and observed a marked decrease in the elastin content of aorta from copper-deficient pigs. Starcher *et al.* (1964) found a decreased quantity of elastin

in the aorta from copper-deficient chicks, and these investigators extended their experiments to show that the effects of copper deficiency on aortic elastin content could be reversed by adding copper to the diet.

The experiments of Starcher *et al.* (1964) with aorta from copper-deficient chicks proved quite valuable in elucidating the function of copper in the production of elastin. First, these authors demonstrated that copper is involved in the production of elastin, rather than in the catabolism of the protein. Second, they observed a marked increase in the quantity of lysine in elastin from copper-deficient chick aorta. This observation drew attention to the work of Partridge (1966), who had earlier proposed a structure for the cross-linkage groups in elastin.

Partridge and co-workers (1964,1966) proposed that the structural integrity of elastin results from the formation of cross-linking substances called desmosine and isodesmosine. This group also obtained experimental evidence which indicated that the cross-linking substances desmosine and isodesmosine in elastin are formed by the condensation of lysine residues in elastin precursors. Thus, the results of Starcher *et al.* (1964) could be explained by assuming that the increased lysine content of elastin from copper-deficient chick aorta resulted from a decreased conversion of lysine to desmosine. This assumption was confirmed by the experiments of Miller *et al.* (1965), who demonstrated that the desmosine content of copper-deficient tissue decreases as the lysine content increases.

The conversion of lysine to desmosine requires oxidative deamination of the ϵ-amino group of lysine residues in preelastin chains. A reaction of this complexity is not likely to occur spontaneously, and this fact sent several investigators in search of a copper-dependent enzyme capable of catalyzing the cross-linking of elastin precursors. Copper-containing amine oxidases capable of deaminating lysine had been isolated previously (Yamada and Yasunobu, 1962; Buffoni and Blaschko, 1964), but first evidence implicating an amine oxidase in aortic elastin cross-linking was presented by Hill *et al.* (1967). These investigators examined amine oxidase activity in chick aorta and found a marked decrease in enzyme activity in aorta from copper-deficient chicks. In addition, Hill *et al.* (1967) maintained aortas in tissue culture and demonstrated that the lysine to desmosine ratio was increased in copper-deficient chick aorta. When a crude preparation of amine oxidase was added to the culture medium, the ratio of lysine to desmosine was decreased in aortas from both copper-deficient and copper-supplemented chicks. These experiments were a strong indication that a copper-dependent amine oxidase is involved in the elastin cross-linking process.

An amine oxidase that catalyzes the oxidation of peptidyllysine to form α-aminoadipic-δ-semialdehyde (allysine) has been isolated from bovine aorta (Rucker *et al.*, 1970) and chick aorta (Harris *et al.*, 1974). This enzyme has been given the name lysyl oxidase to differentiate it from other amine oxidases. Lysyl oxidase contains copper and apparently requires pyridoxal phosphate as a cofactor (Chou *et al.*, 1970).

2.3. Bone Defects

The enzymatic activity of lysyl oxidase is not limited to the aorta. Bone abnormalities have been observed in several species that have been fed copper-deficient diets (Underwood, 1971). Skeletal tissue from copper-deficient animals is characterized by a lack of bone deposition in the cartilage matrix, and the bones from these animals contain more soluble collagen than bones from control animals. The structural integrity of collagen, like elastin, depends upon cross-linking between collagen precursors. The cross-linking process requires lysyl oxidase, which converts lysyl residues into allysine (Pinnell and Martin, 1968; Siegel *et al.*, 1970). Recently, O'Dell *et al.* (1976a) presented experimental results that indicate that lysyl oxidase activity is important for normal development of lung tissue.

To summarize, the defects in connective tissue development that accompany copper-deficiency result from a decrease in the activity of the copper-dependent enzyme, lysyl oxidase. Lysyl oxidase catalyzes the formation of aldehydes (allysine) from peptidyl lysine in pre-elastin and pre-collagen. These aldehyde residues condense to form the covalent cross-links in collagen and elastin (Fig. 3). In copper-deficient tissues, production of the intermediate residues, allysine, is impaired; cross-linking is prevented and the result is a fragility and loss of strength in elastin and collagen.

2.4. Disorders of the Central Nervous System

A debilitating disease has been observed in lambs grazing on copper-deficient pastures in Western Australia and other countries (Underwood, 1971). Lambs affected by this disease walk with a stiff and staggering gait, and the hind quarters sway in incoordinated movement. Many lambs are paralyzed or ataxic at birth and soon die. The condition, known as enzootic neonatal ataxia, can be prevented but not reversed by copper supplementation.

For several years neonatal ataxia was thought to be restricted to sheep but this belief is no longer accepted since the condition has been observed experimentally and under field conditions in several species (Underwood, 1971). Pathological lesions associated with neonatal ataxia vary among species, but lack of myelination of nerve tracts always accompanies the onset of the gross symptoms (O'Dell *et al.*, 1976b).

Because myelin contains a high content of phospholipid, the derangement of myelin accompanying neonatal ataxia was once thought to result from decreased activity of the enzyme α-glycerophosphate acyltransferase (Gallagher *et al.*, 1956). This enzyme catalyzes the attachment of fatty acids to α-glycerophosphate. However, subsequent investigations (Gallagher and Reeve, 1971; DiPaolo and Newberne, 1971) have demonstrated that α-glycerophosphate acyltransferase is not a copper-dependent enzyme.

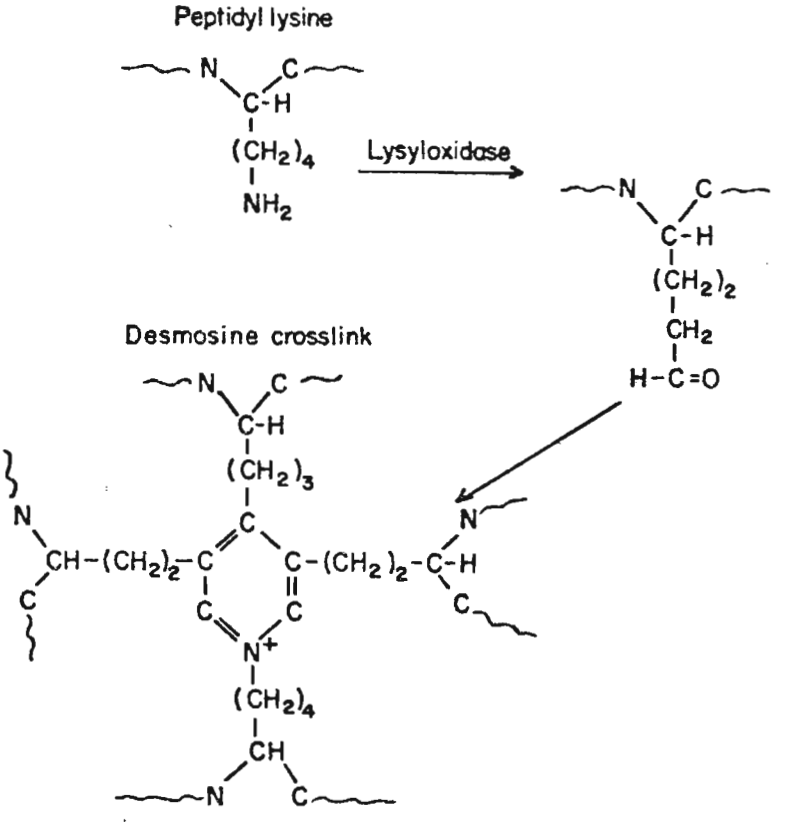

Fig. 3. Schematic illustration of the function of lysyl oxidase in the formation of desmosine crosslinks in elastin.

Several investigators (Mills and Williams, 1962; Prohaska and Wells, 1974) have observed a marked decrease in the copper content and cytochrome c oxidase activity of neural tissue from copper-deficient animals. Cytochrome c oxidase is a copper-dependent enzyme and the terminal oxidase in the respiratory chain of mitochondria. These facts have led to speculation that the primary lesion in neonatal ataxia is the depression of cytochrome c oxidase, which leads to a diminution of aerobic metabolism and a subsequent decrease in phospholipid and myelin synthesis. Although this hypothesis seems tenable, definitive evidence linking the activity of neural cytochrome c oxidase to the production of myelin is lacking. Moreover, yet to be explained are the metabolic effects of the decreased activity in superoxide dismutase and dopamine-β-hydroxylase, two copper-dependent enzymes that are decreased in brain tissue from copper-deficient rats (Prohaska and Wells, 1974).

In view of our lack of knowledge regarding the functions of copper in the central nervous system to attribute the nervous disorder of copper-deficient animals to a derangement of myelin is still premature. O'Dell *et al.* (1976b) have examined the level of neurotransmitters in the brains of ataxic and nonataxic

lambs and found a decreased level of both dopamine and norepinephrine in the brainstem from ataxic animals. Prohaska and Wells (1974) have also detected a significant decrease in the norepinephrine concentration of brains from copper-deficient neonatal rats. The decrease in norepinephrine in copper-deficient tissues can be attributed to a decrease in the activity of the copper enzyme dopamine-β-hydroxylase. The decrease in dopamine concentration is not so easily explained because neither of the enzymes, tyrosine hydroxylase and dopa-decarboxylase, involved in dopamine synthesis is known to be copper-dependent. Whether or not the decreased level of catecholamines in brain tissue from copper-deficient animals leads to nervous disorders remains to be determined. However, some nervous disorders, such as Parkinson's disease, are associated with decreased levels of catecholamines; O'Dell *et al.* (1976b) have pointed out several similarities between Parkinson's disease and neonatal ataxia.

This section may be summarized by pointing out that copper deficiency affects the central nervous system, but the primary lesion that produces the symptoms is unknown. A nervous disorder develops in copper-deficient animals that affects locomotor activity. The ataxic condition is accompanied by hypomyelination of the nerve tract, decreased activity of both neural cytochrome oxidase and superoxide dismutase, and decreased levels of dopamine and norepinephrine in the brain.

2.5. Achromotrichia

Lack of hair and wool pigmentation resulting from copper deficiency has been observed in several species (Underwood, 1971), but is particularly noticeable in black-wooled sheep and rabbits. When black-wooled sheep consume a copper-deficient diet, the wool develops an odd-looking appearance with alternating bands of pigmented and unpigmented fibers. In the rabbit, achromotrichia is a more sensitive index of copper deficiency than is anemia (Smith and Ellis, 1947).

Achromotrichia in copper-deficient animals is probably caused by decreased activity of the copper-dependent enzyme tyrosinase. Tyrosinase catalyzes the conversion of tyrosine to 3,4-dihydroxyphenylalanine (dopa) as well as the oxidation of dopa to dopaquinone. Dopaquinone is then converted through a series of reactions to melanin pigment.

2.6. Steely Wool and Hair

The wool of copper-deficient sheep grows poorly and lacks the crimp characteristic of wool from normal, healthy animals (Underwood, 1971). In addition, the hair of human infants affected with Menkes' syndrome, a genetic disease resulting in malabsorption of copper, has an abnormal, tortuous appearance (Danks *et al.*, 1972b). The wool from copper-deficient sheep, as well as the

hair from Menkes' patients, contain more free sulfhydryl groups than do normal wool and hair (Burley, 1954; Danks *et al.*, 1972b). The presence of these free sulfhydryl groups indicates a reduction in the cross-linking of keratin in the wool and hair fibers, and this accounts for the stringy or steely appearance. The reduction of disulfide bond formation in wool and hair that accompanies copper-deficiency suggests that a copper-dependent enzyme may be required in the process, but no enzyme of this nature has been described to date.

2.7. Reproductive Failure and Hypercholesterolemia

Reproductive failure has been observed in both rats (Hall and Howell, 1969) and chickens (Savage, 1968) as a result of copper deficiency. Simpson *et al.* (1967) have presented evidence that suggests that reproductive failure is caused by defective formation of connective tissue and red cells.

Recently, Allen and Klevay (1976) demonstrated that copper-deficient rats have an increased concentration of plasma cholesterol. These observations suggest that a copper enzyme may be involved in the regulation of cholesterol synthesis or breakdown.

3. Metabolic Disorders Arising from Inborn Errors of Metabolism within the Copper Homeostatic System

3.1. Menkes' Steely Hair Syndrome (Trichopoliodystrophy)

The preceding sections document the widespread role of copper in mammalian metabolism. From these descriptions, one can readily see that an inborn error of metabolism that results in malabsoprtion of copper would be extremely deleterious. Such an inborn error has been discovered in human infants.

In 1962, Menkes and his colleagues (1962) wrote a detailed description of a degenerative disease of the central nervous system. The condition was inherited as a sex-linked recessive trait and the affected males were both physically and mentally retarded and had peculiar white, stubbly hair. Later, O'Brien and Sampson (1966), who first coined the name "Kinky Hair Disease," examined the fatty acid levels in brain tissue from Menkes' patients and found a decreased quantity of docosahexenoic acid, a highly unsaturated fatty acid. At that time, the significance of the peroxidation of lipids was not understood.

In the early 1970s, David Danks and his colleagues in Australia began examining infants suffering from Menkes' kinky hair disease. Acting on the suggestion of Dr. J. M. Gillespie of the Division of Protein Chemistry (CSIRO, in Melbourne), Danks and associates (1972a) examined the copper status of these infants, and a new era in copper metabolism began. In a series of publications Danks *et al.* (1972a,b, 1973b) demonstrated that infants with Menkes' disease

absorb copper poorly and have a decreased concentration of copper in the plasma and liver but an elevated concentration of copper in the intestinal epithelium. These observations have been verified by Walker-Smith *et al.* (1973) and Dekaban *et al.* (1975). In addition, Goka *et al.* (1976) have demonstrated that cultured skin fibroblasts from patients with Menkes' disease contain an elevated concentration of copper.

With the exception of anemia, all the pathological abnormalities associated with copper deficiency in experimental animals have been observed in patients with Menkes' syndrome (Danks, 1975). In fact, the similarity between wool from copper-deficient sheep and hair from Menkes' patients prompted Danks *et al.* (1973a) to suggest that the term "steely hair" be used to describe the hair on these affected infants.

3.1.1. Therapy

As oral copper is poorly absorbed by infants affected with Menkes' syndrome, parenteral copper administration has been used as a therapeutic measure, but the results have been disappointing (Danks, 1975). Copper therapy restores ceruloplasmin and hepatic copper levels to normal, but the symptoms of the disease usually do not improve. This may be because most of the cases reported to date were at least three months old before they were identified and treatment was begun. At this late stage of development, the tissue damage caused by the malabsorption syndrome may be so extensive that it is irreversible. Alternatively, the mutant gene involved in Menkes' syndrome may alter the accessibility of serum copper to tissue cells (Danks, 1975).

Until research establishes exactly how copper is taken up by tissue cells, the methods now being used for copper therapy in Menkes' infants will probably be ineffective. When methods are developed to circumvent the metabolic block of copper uptake by the cells, therapeutic programs will have to be carefully analyzed and designed because Menkes' syndrome is a complex anomaly. For example, some patients exhibit symptoms at birth (Danks *et al.*, 1972a), whereas in others, clinical manifestations appear months after birth (Walker-Smith *et al.*, 1973). These observations suggest the possibility that placental copper transport may be affected, but in varying degrees, by the mutant gene that causes Menkes' disease. If the placenta is affected by the mutant gene, heterozygous carrier females will have to be identified and therapy begun to ensure adequate transport of copper to the developing fetus.

3.1.2. Genetic Heterogeneity

The apparent complexity of the steely-hair syndrome may be partly attributable to genetic heterogeneity. Whereas in most affected infants examined to date, hepatic copper uptake is normal during infusion therapy, in some patients, he-

patic copper uptake is abnormal. Garnica and Fletcher (1975) have observed an abnormally high rate of urinary copper excretion in one patient both during and after copper infusion therapy. As excess copper is normally taken up by the liver and excreted through the bile, this observation suggests that hepatic copper uptake may be affected in some infants with steely-hair syndrome. In addition, Horn *et al.* (1975) examined copper distribution in a male fetus suspected of this inborn error and discovered that the liver was the only tissue that contained less copper than did specimens from control subjects. The copper concentrations of the kidneys, spleen, pancreas, and placenta of the diseased fetus were significantly higher than those from controls. Therefore, the gene mutation that occurs in the steely-hair syndrome may affect the copper transport system of organs other than the intestine.

3.1.3. Animal Model for Menkes' Syndrome

Research on the steely-hair syndrome has been greatly facilitated by the observations of Hunt (1974) who discovered that the sex-linked inherited disorder in mottled mutant (Mo^{br}) mice is nearly identical to the steely-hair syndrome in humans. Hunt found a decreased concentration of copper in livers and brains from Mo^{br} male mice, but the copper concentration of the intestines from the mutant mice was much higher than normal. Ceruloplasmin activity was significantly decreased in the Mo^{br} mice.

Experiments in our laboratory have confirmed the observations of Hunt and also demonstrate that hepatic copper uptake is impaired in Mo^{br} mice. Table II shows the results we obtained when lactating dams were injected with ^{64}Cu and the pups were analyzed 48 h later. In Mo^{br} mice suckling labeled dams, 72% of

Table II. Distribution of ^{64}Cu in Mo^{br} Male Pups, a Heterozygous Female Pup, and Normal Pups Suckling Dams That Had Been Injected with ^{64}Cu

	% Total ^{64}Cu[a]			
Pups[b]	Intestine	Liver	Kidney	Carcass
Male hemizygote (6)	72.2 ± 6.7[f]	2.6 ± 0.5[f]	1.8 ± 0.7	23.4 ± 4.3
Female heterozygote (1)	33.1[f]	13.7[f]	3.5	49.7
Normal homozygote[c] (8)	18.1 ± 1.2	45 .3 ± 1.3	1.0 ± 0.05	35.6 ± 1.7
Normal homozygote[d] (5)	22.8 ± 1.1	49.2 ± 2.4	1.5 ± 0.05	26.5 ± 2.9
Normal homozygote[e] (6)	17.4 ± 1.1	50.6 ± 2.1	4.0 ± 0.1	27.9 ± 2.4

[a]Each value represents mean ± SEM. The values in parentheses refer to number of pups analyzed.
[b]The pups were 5–6 days old when analyzed.
[c]Littermates of the male hemizygotes and female heterozygote.
[d]Pups from a heterozygous dam.
[e]Pups from a homozygous dam.
[f]Differences between the value of the mutant mice and the mean value of the normal control mice were significant at the 1% probability level.

the total radioactivity in the pups was in the intestinal cells, whereas the liver contained only 3% of the radioactivity. In homozygous littermates less than 25% of the radioactivity was found in the intestine and the liver contained 50% of the radioactivity. These results demonstrate that copper accumulates in the intestine of *Mo*^{br} mice, and the results suggest that the copper absorbed from the intestine of these mutants is diverted to extrahepatic tissues.

During our experimentation with *Mo*^{br} mice, we also examined copper metabolism in the *Mo*^{br} heterozygous females. The kidney copper concentration of heterozygous females was significantly higher than that of homozygous females, but there was no difference in liver and brain copper concentrations. In addition, we have observed a marked decrease in copper absorption in heterozygous females (Fig. 4). The observations discussed earlier demonstrate that *Mo* ^{br} mice will have an important role in elucidating the biochemical defects that arise from the lethal mutant gene that is present in infants affected with Menkes' steely hair disease.

3.2. Wilson's Disease (Hepatolenticular Degeneration)

The inborn error of copper metabolism known as Wilson's disease was first described in 1912 (Wilson, 1912). Since that time, volumes of literature have appeared describing various aspects of the disorder. To cover the subject of

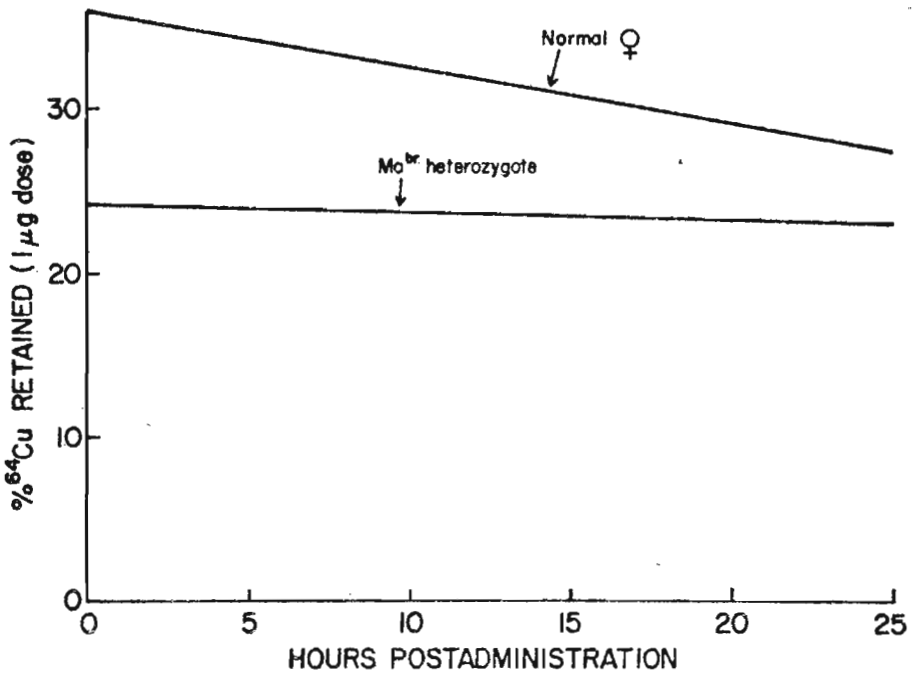

Fig. 4. Absorption and retention of oral ^{64}Cu in normal and heterozygous *Mo* ^{br} female mice. All mice were intubated with 1 μg ^{64}Cu, after which radioactivity was measured hourly in a whole-body counter. Mice in both groups attained a constant rate of isotope excretion 3–5 hr after oral administration. The data depicted are the mean extrapolated values obtained from 10 mice in each group.

Wilson's disease adequately would require a chapter in itself. Therefore, the comments of this section will be brief and directed toward informing the uninformed regarding the pathogenesis, pathology, and treatment of Wilson's disease. For more comprehensive discussions of the disease, the reader is referred to several excellent reviews (Sass-Kortsak, 1965; Walshe, 1966; Bearn, 1972; Scheinberg and Sternlieb, 1975).

The mutant gene in individuals with Wilson's disease produces a yet-undiscovered biochemical defect that results in excess retention of hepatic copper. Most affected patients have a decreased concentration of plasma ceruloplasmin and the excretion of copper into the bile is impaired. Thus, the defect apparently involves the mechanisms that regulate the passage of copper into the ceruloplasmin-synthesizing and biliary-excretion pathways.

After several years of accumulating copper, the capacity of the liver is eventually exceeded, and copper begins to diffuse into the plasma and fluids of extrahepatic tissues. At this point in the progression of the disease, massive necrosis can be detected in the hepatic parenchymal cells, and the first clinical symptoms begin to appear in the form of liver dysfunction.

If the untreated patient survives the liver disease, pathological changes resulting from excess copper eventually appear in the central nervous system, kidneys, and cornea. At this stage, neurological and psychiatric symptoms may appear, and renal function is impaired. If untreated, the manifestations of copper toxicity result in death at an early age.

Fortunately, the manifestations of Wilson's disease can be arrested and prevented by drug therapy. The drug now being used most successfully is D-penicillamine (β,β-dimethylcysteine), which produces a marked increase in the urinary excretion of copper. Treatment with penicillamine results in a dramatic recovery of the affected patients and continuous therapy expands the lifespan of individuals who possess this mutant gene that alters copper homeostasis in the liver.

4. Metabolic Disorders Resulting from an Absence of a Specific Copper Enzyme

4.1. Albinism

The only known inborn error of metabolism that can be linked to a copper-dependent enzyme is albinism. A genetically acquired absence of the enzyme tyrosinase results in albinism, which is characterized by the complete lack of pigmentation in the eyes and integument. Tyrosinase is essential in the pigmentation process because this enzyme catalyzes the first two steps in the synthesis of melanin pigment from tyrosine. Mammals that lack pigment do not have the capability to filter the sun's ultraviolet rays, which penetrate the integument and cause molecular havoc.

4.2. Unidentified Disorders and "Enzymes Looking for a Disease"

Although only one genetic disease has thus far been linked to a copper enzyme, this is not to say that others do not exist. In view of the number of copper enzymes present in mammalian systems, there is a good possibility that some of the diseases of unknown etiology will some day be linked to a copper enzyme.

Some of the copper enzymes that have not been associated with a specific pathological defect include cytochrome c oxidase, the terminal enzyme in the mitochondrial electron transport system; superoxide dismutase, the enzyme that catalyzes the dismutation of toxic superoxide anions; dopamine-β-hydroxylase, the enzyme that catalyzes the conversion of dopamine to norepinephrine; and uricase, the enzyme that catalyzes the oxidation of uric acid. Careful analysis of the pathological defects that accompany copper deficiency will probably lead to association of some of these enzymes with specific pathological defects. However, some of these enzymes may bind copper very tenaciously or have a slow turnover rate. Also, some of the enzymes may be present in a large excess. These characteristics would preclude use of experimental copper deficiency for associating the enzyme with a specific defect. In these cases, the effects of loss of the enzyme can only be determined when a gene mutation occurs that abolishes synthesis of the enzyme.

5. Relevance of Research on the Role of Copper in Metabolism

After reviewing the list of pathological defects that result from copper deficiency, the reader is left with an impression of the importance of copper in growth and development. However, the foregoing discussion in this chapter would be purely academic without at least a brief discussion of the relevance of these research observations.

The recognition of the many pathological anomalies associated with copper deficiency will undoubtedly aid in the diagnosis of ailments that arise when dietary copper is low or unavailable. Support for this statement has already been provided in the section describing Menkes' steely hair syndrome. The etiology of this inborn error was discovered when the symptoms of the disease were compared with the symptoms that occur in experimental copper deficiency.

Recognizing the symptoms of copper deficiency is not limited to use as a diagnostic tool for elucidating the etiology of inborn errors of metabolism. As mentioned in the second section, copper deficiency occurs in animals grazing on soils where copper is low or unavailable. Knowledge of the symptoms of copper deficiency will enable rapid diagnosis and alleviation of the nutritional deficiency in problem areas. The economic implications of this source of knowledge are obvious.

Recognition of the symptoms of copper deficiency has been and will continue to be extremely important in maintaining the health and well-being of humans. Despite suggestions to the contrary copper deficiency does occur in human beings. As early as 1931, Josephs (1931) detected copper deficiency in infants. More recently, the symptoms of copper deficiency have been observed in infants fed low-copper milk diets (Cordano *et al.*, 1964), in premature infants (Al-Rashid and Spangler, 1971), in infants nourished by total parenteral alimentation (Karpel and Peden, 1972) and in adults nourished by total parenteral alimentation (Dunlap *et al.*, 1974). The latter two instances occurred in a clinical situation; recognition of the symptoms of copper deficiency enabled the clinicians to diagnose and alleviate the problem.

Our present knowledge of the nutritive value of foods suggests that most diets contain an adequate level of copper. However, to assume that all factions of society are now ingesting or will continue to ingest an adequate level of dietary copper is presumptuous. Copper pipe, once a source of dietary copper, is being replaced by plastic products; diets are continually becoming more refined; and food faddism is running rampant. If changes in our lifestyle do affect copper nutriture, our knowledge of the role of copper in mammalian metabolism will prove invaluable in diagnosing and preventing the occurrence of severe deficiency syndromes.

6. Summary

Although absolutely essential for normal growth and development but, in excess, copper is extremely toxic. Living systems have developed methods for conserving copper in the body while preventing excess accumulation.

Copper deficiency results in anemia, defects in connective tissue, abnormal functioning of the central nervous system, lack of pigmentation, poor hair or wool development, infertility, and poor growth. Although several copper enzymes have been identified, only a few have been linked to specific pathological defects that accompany copper deficiency.

Two genetic disorders that affect the balance of copper have been discovered, but the biochemical defects have not been described. Studies on the metabolism of copper are still in their infancy, and without doubt, future volumes of *Advances in Nutritional Research* will contain many significant revelations with regard to the biochemistry and physiology of this fascinating element.

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